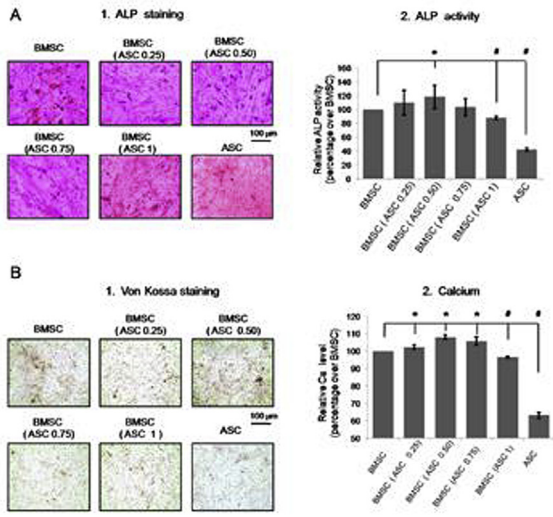


Fig. 1. **Effects of ASCs cultured in transwell on BMSCs**. ASC 0.25, 0.50, 0.75, I means that $0.25 \times 10^5$, $0.5 \times 10^5$, $0.75 \times 10^5$, and $1.0 \times 10^5$ ASCs respectively were cultured in a transwell insert over $10^5$ BMSCs on the lower well. (A) ALP staining, quantification of ALP activity and the ALP mRNA expression from RT-qPCR after 14 days. (B) Von Kossa staining, quantification of calcium contents after 14 days. All values are shown as percentages over the control (BMSCs without ASCs). Bar represents mean ± SD, $p < 0.05$: * significantly greater than the control; # significantly lower than the control.

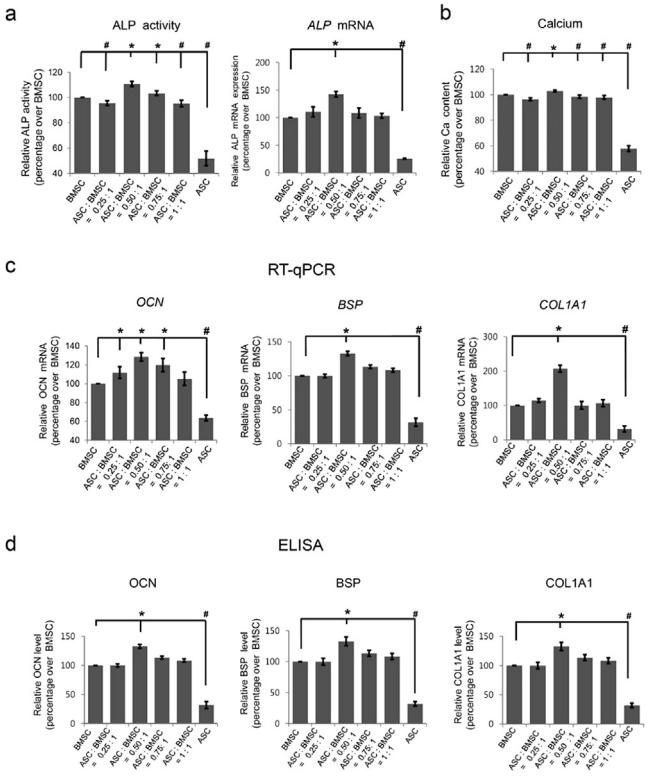


Fig. 2. **Effects of ASCs in mixed coculture with BMSCs**. The number of BMSCs is fixed at $1.0 \times 105$. "ASC : BMSC = 0.25: 1" means that $0.25 \times 105$ ASCs were cocultured with $1.0 \times 105$ BMSCs. All values are shown as percentages over the control (BMSCs without ASCs). (a) ALP activity (b) Calcium contents (c) RT-qPCR for osteogenic markers (d) ELISA for osteogenic markers after 14 days. Bar represents mean ± SD. N = 3 for all panels. $p < 0.05$, *significantly greater than the control; # significantly lower than the control.

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## MEAN ARTERIAL PRESSURE ASSOCIATES WITH UNCOUPLED REMODELING OF SUBCHONDRAL BONE IN PATIENTS WITH KNEE OSTEOARTHRITIS

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**Purpose**: Hypertension frequently coexists with knee osteoarthritis (OA), and high blood pressure was proven to be a predictor of bone loss. Our previous animal model study found that high concentration of TGF-β1 induced formation of mesenchymal stem cells (MSCs) clusters, leading to uncoupled bone remodeling in subchondral bone and inducing pathological development of OA. This study investigates whether the level of mean arterial pressure (MAP) is correlated with the extent of TGF-β1 signaling and subchondral bone remodeling in knee OA patients.
**Methods**: A total of 70 patients undergoing arthroplasty surgery for primary OA were recruited and divided into two groups according to their pre-operative MAP: normal (n = 33) or elevated (n = 37). The patients' preoperative clinical and radiological evaluations were recorded. The tibial plateau were collected during the operation and processed for evaluation using micro-CT and histology. Biomarkers of MSC (Nestin), osteoclast (Trap), osteoblast (Osterix and Osteocalcin), and TGF-β1 signaling (pSMAD2/3) were detected using immunohistochemistry.
**Results**: There were no significant differences of age, body mass index (BMI) or mechanical alignment detected between the two groups. Patients with elevated MAP presented lower fraction of bone volume (BV/TV) (30.71 ± 4.78%) and trabecular number (Tb.N) (1.88 ± 0.42) of medial subchondral bone compared with those of normal MAP (BV/TV: 36.97 ± 7.51%, $p = 0.005$; Tb.N: 2.25 ± 0.47, $p = 0.013$), yet higher BV/TV (21.24 ± 4.10%) and Tb.N (1.40 ± 0.33) of lateral side compared with those of normal MAP (BV/TV: 17.76 ± 4.05%, $p = 0.004$; Tb.N: 1.22 ± 0.23, $p = 0.02$) and the correlation of MAP with BV/TV and Tb.N still exits after adjusting for age, sex, BMI and mechanical alignment ($p < 0.05$). Histologically, the elevated MAP group showed higher OARSI scores of both medial(23.2 ± 1.8) and lateral(10.0 ± 1.9) side than the normal MAP group(medial 21.6 ± 0.9, $p = 0.081$;lateral 6.2 ± 1.3, $p = 0.001$). More TRAP positive cells were present in medial subchondral bone of the elevated MAP group (111 ± 6/ mm ‡) compared with the normal MAP group (56 ± 3/mm ‡, $p < 0.05$). However, less Nestin (23 ± 3/mm ‡), pSMAD2/3 (823 ± 174/mm ‡), Osterix (208 ± 33/mm ‡) and Osteocalcin (31 ± 4/B.Pm) positive cells were observed on the medial side of elevated MAP group compared with the normal MAP group (Nestin: 31 ± 4/mm ‡, $p < 0.05$; pSMAD2/3: 995 ± 214/mm ‡, $p < 0.05$; Osterix: 247 ± 54/mm ‡, $p < 0.05$; Osteocalcin: 42 ± 5/B.Pm, $p < 0.05$), which is on the contrary to the changes on the lateral side.
**Conclusions**: Our data show that elevated MAP is associated with uncoupled remodeling of subchondral bone in patients with advanced knee OA, indicated by paradoxical changes of bone between the medial and lateral side. Furthermore, uneven local recruitment of MSCs in subchondral bone mediated by TGF-β1 signaling may be involved in this pathological development. These findings provide a possible underlying mechanism of the association between hypertension and pathological development of OA, and might guide us to more individualized OA treatment.

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## HUMAN SKELETAL MUSCLE-DERIVED PDGFRα+ CELLS FORMED HETEROTOPIC OSSIFICATION

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**Purpose**: Heterotopic ossification (HO) is one of the most common complications after joint surgery and can compromise outcomes of patients. HO is defined as the formation of ectopic bone in soft tissue